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The Role of CD8+T Cells in the Immune Response to Trypanosoma Cruzi in Mice Held at Elevated Environmental Temperature

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THE ROLE OF CD8+ T CELLS IN THE IMMUNE RESPONSE TO *TRYPANOSOMA CRUZI* IN MICE HELD AT ELEVATED ENVIRONMENTAL TEMPERATURE

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THE ROLE OF CD8+ T CELLS IN THE IMMUNE RESPONSE TO *TRYPANOSOMA CRUZI* IN MICE HELD AT ELEVATED ENVIRONMENTAL TEMPERATURE

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When maintained at 36°C, *T. cruzi* infected C3H mice survive an infection that is lethal to mice maintained at room temperature. To study the role of CD8+ T cells in this phenomenon, anti-CD8 monoclonal antibody (MAb) was used to deplete CD8+ T cells *in vivo.* An IgG2a-producing rat hybridoma (designated 53-6.72) was adapted to serum free medium, and anti-CD8 MAb was purified from culture supernatant by ammonium sulfate precipitation. Mice were injected intraperitoneally with 200μg of MAb for three consecutive days. On the fifth day, the result of the *in vivo* depletion of CD8+ T cells was determined by an indirect immunofluorescence assay and was found to be approximately 95%. Mice were then infected with 10^3 blood-form trypomastigotes. Parasitemia and longevity were monitored. The results indicated that anti-CD8 MAb treated infected mice developed higher parasitemia levels and higher mortality as compared to normal IgG treated mice at both temperatures. Parasites began to appear in the peripheral blood of anti-CD8 MAb treated infected mice on approximately day 13 of infection when mice were maintained at

room temperature but not until approximately day 20 of infection when anti-CD8 treated infected mice were held at elevated environmental temperature. The parasitemia levels of anti-CD8 MAb treated infected mice held at room temperature reached a peak at 1.12 X 10^7 parasites per ml of blood, whereas those held at elevated environmental temperature reached a peak parasitemia of 1.10 X 10^7. Parasitemia levels in normal IgG-treated infected mice at room temperature reached a peak of 1.02 X 10^7 parasites per ml of blood whereas parasitemia levels in normal IgG treated infected mice held at elevated environmental temperature only reached 3.1 X 10^5 per ml of blood by day 53 of infection. All anti-CD8 MAb treated infected mice at room temperature died by day 27 of infection, whereas those held at elevated environmental temperature died by day 35. All normal IgG-treated infected mice at room temperature died by day 36 of infection. However, all normal IgG-treated infected mice held at elevated environmental temperature were still alive by day 53 of infection. The results of this study suggest that CD8+ T cells play a significant role in the immune response to *T. cruzi* in mice maintained at elevated environmental temperature. Depletion of CD8+ T cells from mice by administration of anti-CD8 MAb resulted in an abrogation of the protective effects of elevated environmental temperature on *Trypanosoma cruzi* infection.

INTRODUCTION

Background

Trypanosoma cruzi, the causative agent of Chagas' disease, is an obligate intracellular protozoan parasite. This parasite infects humans and other mammals primarily in Latin America but also in North America. It is estimated that 13 million people are infected with *T. cruzi* in the areas of South and Central America. During the acute stage of the disease, fever, headache, and prostration may develop within 1-3 weeks after infection. Enlargement of the liver and spleen as well as myocardial damage may follow. In chronic cases, cardiac and gastrointestinal symptoms may be involved. Presently, there is no effective treatment available for Chagas' disease (Bogitsh and Cheng, 1990).

Life cycle of *T. cruzi*

The agents of transmission and intermediate hosts of *T. cruzi* are blood-sucking bugs in the family Reduviidae (kissing bugs) in whose hind gut the metacyclic trypomastigote stage develops. When a bug takes a blood meal from its vertebrate host, it simultaneously defecates. As a result of scratching or rubbing, the parasites enter the wound, eyes, or other mucous membranes of the host. Trypomastigotes immediately enter the host cells at the wound site where they transform into the intracellular amastigote form. The amastigotes divide by longitudinal binary fission

inside the host cell. The amastigotes then transform into trypomastigote stages which are subsequently released from the cell. Trypomastigotes in turn invade other cells, or enter the bloodstream. (Bogitsh and Cheng, 1990). Figure 1 shows the life cycle of *T. cruzi* in humans and in the insect *Panstrongylus sp.* (kissing bug).

The immune response to *T. cruzi* infection

T. cruzi is capable of infecting a wide variety of cell types in humans and other mammals including macrophages, smooth and cardiac muscle, neurons and fibroblasts. Nearly every aspect of the immune system has been shown to play a significant role in resistance to *T. cruzi* infection (Kierszenbaum and Pienkowski, 1979; Trischmann and Bloom, 1980; Kuhn, 1981). It is known that the protective immune response to *T. cruzi* in mice and in humans involves the production of antibodies (Kretti and Brener, 1976; 1982), helper and effector functions of CD4+ T cells (Rottenberg et al., 1988; Araujo, 1989), cytotoxic activity of CD8+ T cells (Tarleton, 1990; Tarleton, 1992) and killing by natural killer cells (Rottenberg et al, 1988) as well as the regulatory function of cytokines (Wirth et al., 1985; Reed, 1988; Wirth et al., 1989; Silva et al, 1992). Macrophages may also be activated to destroy this intracellular parasite (Piuvezam et al., 1993).

The role of CD8+ T cells in *T. cruzi* infection

CD8+ T cells are those cells carrying the CD8 surface molecule that functions together with the T-cell receptor complex. These cells can be activated by antigens presented with class I major histocompatibility complex (MHC) molecules.

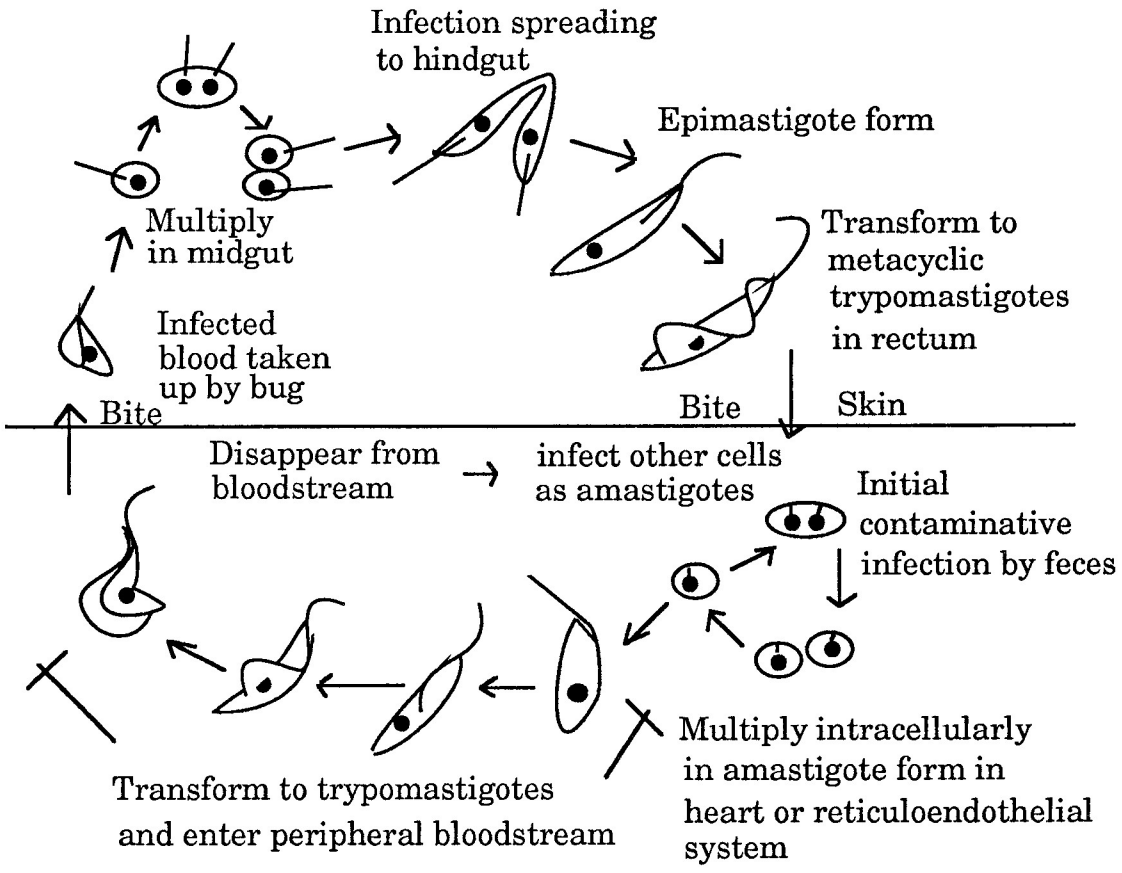

Figure 1. Life cycle of *Trypanosoma cruzi* in humans and in the insect *Panstrongylus*
(Adapted from Bogitsh and Cheng, 1990)

CD8+ T cells have long been known to be involved in viral infection due to their cytotoxic and suppressor function (Zinkernagel and Doherty, 1977). More recently, CD8+ T cells have been implicated in the anti-bacterial (Chiplunkar et al., 1986) and anti-parasite immune response (Pearson et al., 1982; Kumar et al., 1988; Khan et al., 1988; Tarleton, 1992). CD8+ T cells also play a role in the immune response to the following protozoal parasites: *Leismania major* (Titus et al., 1987), *Plasmodium sp.* (Schofield et al., 1987; Weiss et al., 1988), *Toxoplasma gondii* (Suzuki and Remington, 1988), *Theileria parva* (Pearson et al., 1982), and *Trypanosoma cruzi* (Tarleton, 1992). The relative role of CD8+ T cells in the immune response to *T. cruzi* infection was studied by the *in vivo* administration of an anti-CD8 monoclonal antibody. This treatment was found to increase susceptibility in mice to *T. cruzi* infection in both resistant and highly susceptible mouse strains and resulted in increased parasitemia levels and mortality (Tarleton, 1990; 1991). Another study reported that ß2-microglobulin-deficient mice which lack class I MHC molecules failed to resist *T. cruzi* infection (Tarleton, 1992). The cytotoxic activity of CD8+ T cells in the immune response to *T. cruzi* has also been demonstrated (Nickell, 1993). Splenic T cells from mice chronically infected with *T. cruzi* were shown to express high levels of cytolytic activity when stimulated with irradiated *T. cruzi* infected macrophages. The CD8+ T cells acted in an antigen-specific and MHC-restricted manner when target cells were lysed (Nickell, 1993). A study of CD8+ T cells in the immune response to *T. cruzi* infection in mice at the level of the infected cell also showed evidence that

CD8+ T cells are crucial. CD8+ T cells therefore play a major role in the anti-parasite immune response during *T. cruzi* infection.

Effect of elevated environmental temperature on *T. cruzi* infection

T. cruzi -infected mice or rats maintained at elevated environmental temperature survive an otherwise lethal infection (Kolodny, 1939; 1940; Trejos et al., 1965; Amrein, 1967; Marinkelle and Rodriguez, 1968; Otieno, 1972; 1973; Anderson and Kuhn, 1989). The effect of elevated environmental temperature on the host's resistance to *T. cruzi* infection in mice is reflected not only by decreased parasitemia levels and increased longevity, but also by enhanced antibody responses (Marinkelle and Rodriguez, 1968; Smith et al., 1978; Saririan and Nickerson, 1982; Jampel et al., 1983; Anderson and Kuhn, 1989; Dimock et al., 1991), and enhanced T helper cell responses to heterologous antigens (Anderson and Kuhn, 1989). The parasite-specific response of T helper cells in *T. cruzi*-infected mice held at 36°C is also enhanced (Anderson and Kuhn, 1989). However, from the results of experiments reported, it appears that during the acute phase of infection, parasite-specific antibodies do not play a significant role in the observed protective immunity. Levels of parasite-specific antibodies in infected mice held at 36°C are actually lower than in mice held at room temperature because of a deficit in parasite antigen due to lowered antigen dose as a result of decreased parasitemia levels (Anderson and Kuhn, 1989).

Several investigators have observed that protection against infection with *T. cruzi* can be obtained by the adoptive transfer of spleen cells or the

passive transfer of serum from immunized mice, or from mice which have otherwise survived acute infection (Marinkelle and Rodriguez, 1968; Kuhn and Durum, 1975; Kierszenbaum and Howard, 1976; Nogueira et al., 1981; Rowland and Ritter, 1984; Rottenberg et al., 1990). Anderson reported that transfer of either spleen cells or serum from mice that survived infection at 36°C led to decreased parasitemia and increased longevity in recipient mice held at room temperature (Anderson and Kuhn, 1989). These results indicate that cellular and/or humoral responses play a role in the immune response to *T. cruzi* in mice held at 36°C. The beneficial effects of fever therapy have also been reported in the treatment of humans with acute Chagas' disease (Acuna et al., 1963).

In the present study, cell depletion by anti-CD8 monoclonal antibody treatment was used to study the contribution of T cells bearing the CD8 marker (classical cytotoxic/suppressor T cells) to *T. cruzi* infection in a highly susceptible mouse strain (C3H) held at elevated environmental temperature. *In vivo* depletion of CD8+ T cells has been a highly successful approach for determining the contribution of this cell type to the immune response in a variety of different experimental systems. The *in vivo* injection of monoclonal antibodies results in the almost complete depletion of CD8+ T cells from the spleen (Kruisbeek, 1991).

The C3H (susceptible) inbred mouse strain has been useful in the elucidation of many of the immunological aspects of this parasite/host association. When susceptible C3H mice are infected with the Brazil strain of *T. cruzi,* their immune responsiveness is significantly suppressed, and

this suppression extends to both non-specific and parasite-specific responses (Cunningham and Kuhn, 1980; Kuhn, 1981). In addition, parasitemia levels in *T. cruzi*-infected C3H mice reach as high as 1.4 X 10^7 parasites per ml of blood and infected mice die by day 35 of infection (Anderson and Kuhn, 1989; Dimock et al., 1991). Maintenance of *T. cruzi* infected C3H mice at 36°C not only increases longevity and decreases parasitemia levels but also significantly enhances both parasite-specific and non-specific immune responses (Anderson and Kuhn, 1989).

MATERIALS AND METHODS

Mice

C3HeB/FeJ inbred mice (Jackson Laboratory, Bar Harbor, Maine), 8-10 weeks old at time of infection, were used in this study. All mice were kept at room temperature (26°C) for 2 weeks prior to use. After infection, mice were either maintained at room temperature or were placed in an environmental chamber at 36°C. Age-matched groups of noninfected mice were also held at either room temperature or 36°C for the course of the experiments. Food and water were provided *ad libitum*. All mice were maintained and used in accordance with NIH and local IACUC guidelines (All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated).

Parasites

The parasites used in this study were of a Brazil strain of *T. cruzi*. These parasites were maintained in the laboratory as stock infections in C3HeB/FeJ mice (blood-form trypomastigotes, BFTs). Mice were infected intraperitoneally with 10^3 BFTs in 0.2 ml of Dulbecco's phosphate buffered saline (DPBS).

Cell culture

The rat-hybridoma 53-6.72 cell line (kindly provided by Dr. Rick Tarleton, Department of Zoology, The University of Georgia) was maintained in RPMI-1640 and HEPES buffer supplemented with 10% fetal bovine serum, penicillin G (100 units/ml), and streptomycin (100 mg/ml) (complete RPMI-1640). Alternatively, the 53-6.72 cell line was adapted to serum-free medium (Tarleton and Bryer, 1991). The cells were then maintained in 75 cm^2 T-flasks at 36°C with 5% CO2 and high humidity under sterile culture conditions.

Purification of monoclonal antibody

Anti-CD8 monoclonal antibody from hybridoma culture supernatants of RPMI 1640 medium was purified by ammonium sulfate precipitation followed by protein G-sepharose affinity chromatography. Protein G has been shown to be very useful for the purification of rat IgGs of all subclasses (Andrew and Titus, 1991). Anti-CD8 monoclonal antibodies from serum-free media were directly purified by ammonium sulfate precipitation as follows.

Culture supernatant containing anti-CD8 monoclonal antibody was harvested by centrifugation at 13,000 X g, and the supernatants were placed into a flask on ice. Saturated ammonium sulfate was added very slowly while the flask was gently agitated. The flask was held at 4°C overnight and the resulting precipitates were centrifuged for 1 hour at 13,000 X g, 4°C. The supernatnants were discarded and the precipitate was dissolved in several milliliters of phosphate buffered saline (PBS). The dissolved

precipitate was then dialyzed against a large volume of PBS for 24 to 48 hours at 4°C. The buffer was changed frequently (4-6 hours) during dialysis. After ammonium sulfate purification, protein G affinity chromatography was used to further purify monoclonal antibodies precipitated from complete RPMI 1640 medium. No further purification was required for hybridoma cell culture supernatants prepared from serum free medium.

A immobilized protein G gel column was prepared by pipetting the resin into the column followed by equilibrating with 5 volumes of binding buffer (0.1 M sodium acetate, pH 5.0). The antibody sample was then diluted 1:1 with binding buffer before adding to the equilibrated column. The sample was allowed to flow completely through the column. The protein G column was washed with 6-10 column volumes of binding buffer. The bound IgG was eluted with 10 ml elution buffer (50mM glycine HCl buffer, pH 2.5-2.8). The eluate was collected in 1 ml fractions, and 100 µl of 500mM Tris buffer, pH 7.6 was added to each tube to restore pH. The protein content of purified monoclonal antibody preparations were determined by Bradford assay (Bradford, 1976). Purified monoclonal antibody was standardized to a final concentration of 1 mg/ml in PBS and aliquotes were stored at -70°C.

In vivo depletion of CD8+ T cells

Fourteen mice were injected intraperitoneally for three consecutive days with 200 µg of purified anti-CD8 monoclonal antibody. Fourteen control mice were injected with an equivalent concentration of normal rat IgG. To confirm the specificity of depletion of CD8+ T cells, spleen cells from one

mouse from each experimental group were analyzed for the presence of the CD8 glycoprotein on day five by an indirect immunofluorescence assay followed by direct cell counting on an Olympus BH-2-RF1 fluorescence microscope. Mice were then infected with 10^3 blood-form trypomastigotes (BFTs) of *T. cruzi*. One-half of the mice were placed immediately at room temperature and the other one-half at 36°C. Groups of uninfected mice injected with anti-CD8 monoclonal antibody were also monitored for control purposes. To maintain the CD8-depleted condition, mice were given 200 μg injections of monoclonal antibody weekly throughout the course of the experiment.

Immunofluorescence assay

Mice were killed by anesthetizing in a jar containing ether-soaked cotton. The abdomen of each mouse was doused with 70% ethanol. A cut was made through the loose skin in the inguinal region. The skin was pulled back on either side of the cut until the peritoneal wall was exposed. The peritoneal wall was doused with 70% ethanol to remove loose hair, and the peritoneal wall was lifted over the spleen with sterile forceps. A large U-shaped cut was made on the left side around the spleen. The peritoneum was folded back and the spleen was lifted with the forceps. Connective tissue was removed with sterile scissors. The spleen was placed into a small petri dish containing RPMI-1640. The spleen was then minced with large forceps. To obtain a single-cell suspension, spleen cells were drawn up into a 5cc syringe through a 22 gauge needle and expelled into a 15cc tube through a 26 gauge needle. Erythrocytes were then lysed by hypotonic

shock.

Twenty µl of the spleen cell suspension was diluted in 380 µl of trypan blue solution and loaded into a hemacytometer using a micropipet. The number of viable cells was determined.

A 100 µl spleen cell suspension at a concentration of 1 X 10^7 cells/ml in RPMI-1640 was incubated with 10 µg of purified monoclonal antibody at 4°C for 30 min. The cells were then washed with RPMI three times and incubated with 5 µg of fluorescein isothiocyanate (FITC)-labelled goat anti-rat IgG at 4°C for 30 min. The cells were then washed three times, and the positive cells were determined on an Olympus BH-2-RF1 fluorescence microscope.

Calculation of percent depletion

The percent of depletion of CD8+ T cells was calculated by the following formulation:

Percent Depletion=(% positive cells of normal IgG-treated mice - % positive cells of negative control) - (% positive cells of depletion mice - % positive cells of negative control) / (% positive cells of normal IgG-treated mice-% positive cells of negative control) X 100%

Parasitemia and mortality

The effect of CD8+ T cell depletion on the course of *T. cruzi* infection in mice maintained at room temperature and elevated environmental temperature was determined by monitoring both parasitemia levels and longevity. Parasites were determined by hemacytometer counting of 4µl of tail vein blood diluted in 96 µl sterile saline solution beginning at day 7 of

infection and then continuing every three days throughout of the course of infection. Mice were monitored daily to record physical appearance and mortality.

RESULTS

Determination of concentrations of anti-CD8 monoclonal antibody

The concentration of anti-CD8 monoclonal antibody was determined by Bradford assay (Bradford, 1976). The standard curve that was generated with dilutions of bovine serum albumin had an R value for linear regression analysis of 0.990 (Figure 2). The concentration of purified monoclonal antibody was calculated with the linear regression equation and was found to be approximately 1 mg/ml.

Result of depletion of the CD8+ T cells

C3H mice were injected intraperitoneally with 200 μg anti-CD8 monoclonal antibody on three consecutive days. Two days following the last injection, the percentage depletion of the CD8+ T cells in the spleen was determined by an indirect immunofluorescence assay on a single experimental animal from each group. Figure 3 shows the results of anti-CD8 monoclonal antibody depletion of CD8+ T cells. There were approximately 25% CD8+ T cells in normal rat IgG-treated mice, whereas in anti-CD8 treated mice there were approximately 6% CD8+ T cells. When background values were substracted from experimental values, the degree of depletion in anti-CD8 treated mice was found to be approximately 95% as compared to normal rat IgG-treated control mice.

Effect of CD8+ T cell depletion on parasitemia levels in infected mice

C3H mice were infected with 10^3 blood form trypomastigotes following the depletion of CD8+ T cells. Anti-CD8 treated infected mice showed higher parasitemia levels than normal rat IgG-treated infected mice at either temperature (Figure 4). Blood-form trypomastigotes began to appear in the peripheral blood of anti-CD8 treated infected mice on day 13 of infection when mice were maintained at room temperature. Parasites were detectable in the blood of anti-CD8 treated infected mice on day 20 of infection when mice were held at elevated environmental temperature. The mean parasitemia level of anti-CD8 treated infected mice held at room temperature reached 1.12×10^7 parasites per ml of blood, whereas normal IgG-treated infected mice maintained at room temperature had a parasitemia level 1.0×10^7 parasites per ml of blood. Similar trends were observed in anti-CD8 treated infected mice maintained at elevated environmental temperature. Anti-CD8 treated infected mice reached a mean parasitemia level of 1.10×10^7 parasites per ml of blood by day 35 of infection. Normal IgG-treated infected mice maintained at 36°C showed a peak parasitemia level of only 3.10×10^5 parasites per ml of blood by day 53 post-infection.

Effect of CD8+ T cell depletion on mortality in infected mice

Anti-CD8 treated infected mice showed significant mortality as compared to normal IgG-treated infected mice maintained at the same temperature (Figure 5). Anti-CD8 treated infected mice held at room temperature died approximately one week earlier than anti-CD8 treated

infected mice maintained at elevated environmental temperature. Anti-CD8 treated infected mice held at room temperature died between days 26 and 28 of infection with a mean survival time of 27 days post-infection. Anti-CD8 treated infected mice maintained at elevated environmental temperature died between 34 and 36 days of infection with a mean survival time of 35 days post-infection. Normal IgG-treated infected mice held at room temperature died by day 36 post-infection. However, normal IgG-treated infected mice held at elevated environmental temperature were still alive by day 53 post-infection.

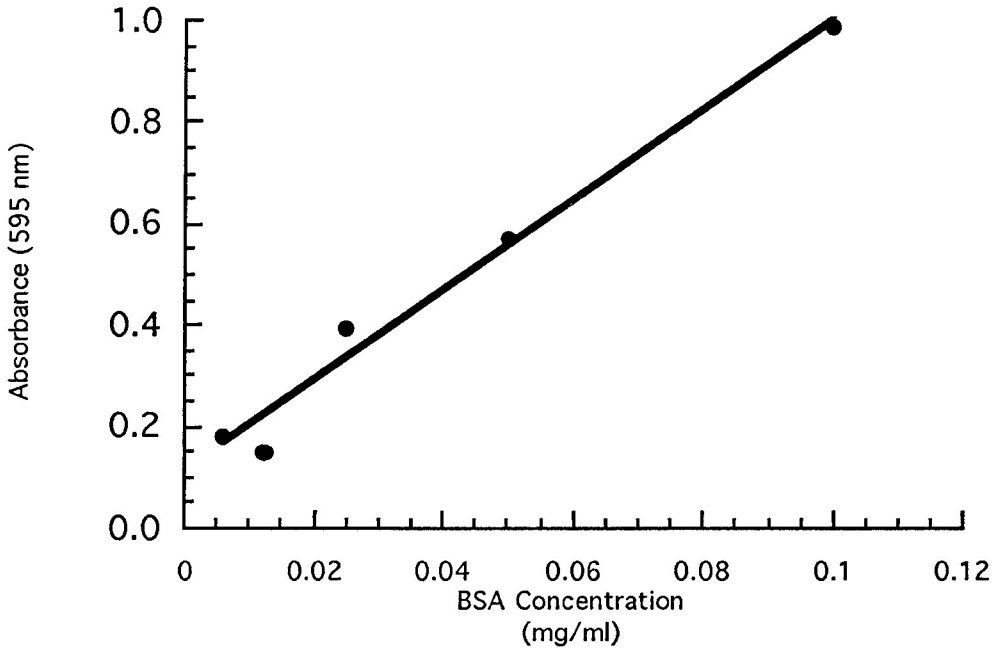

Figure 2. Protein standard curve. Bovine serum albumin was used as the protein standard. The concentration of anti-CD8 monoclonal antibody was determined by the linear regression. The prediction equation is $y = 0.110 + 8.903x$. The R value is 0.990.

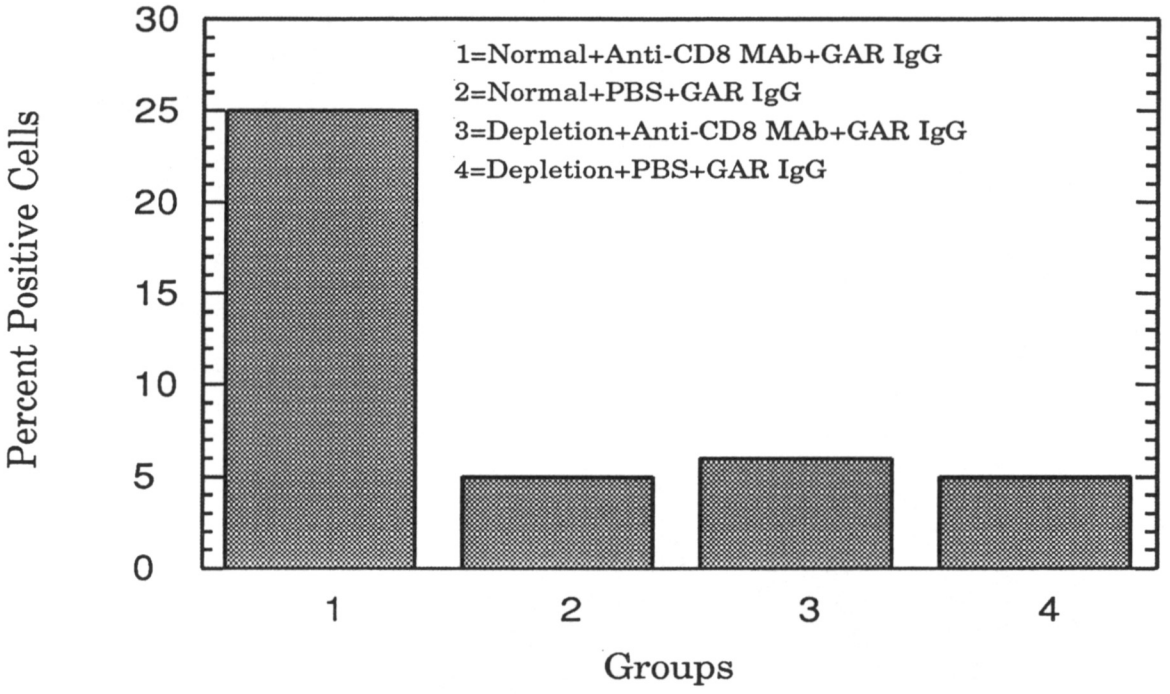

Figure 3. CD8+ T cell depletion assay. 200 μg anti-CD8 monoclonal antibody or 200 μg normal rat IgG were used to inject C3H mice on three consecutive days. On day five, the percentage of CD8+ T cells was determined by an indirect immunofluorescence assay. Percent depletion = (25%-5%) - (6%-5%) / (25%-5%) X 100% = 95%. GAR IgG = fluorescein-conjugated goat anti-rat IgG.

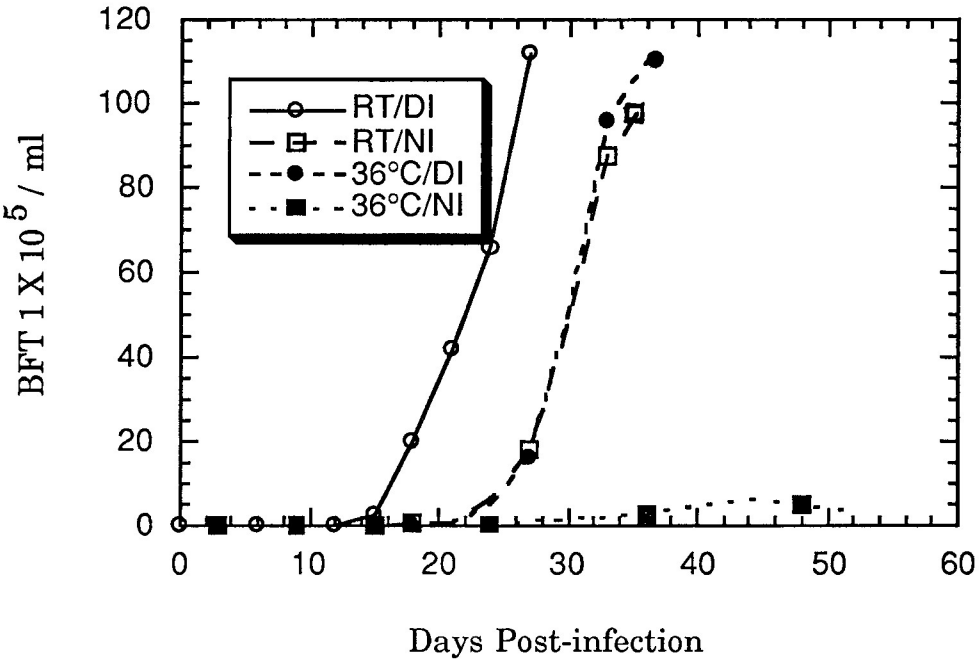

Figure 4. Effect of CD8+ T cell depletion on parasitemia levels. Effect of anti-CD8 and normal IgG treatment on parasitemia levels in *T. cruzi* infected mice maintained at either room temperature or 36°C. Two groups of five C3H mice were injected with 200 μg anti-CD8 monoclonal antibody and 200 μg normal rat IgG for three consecutive days. On day five, mice were infected with 10^3 BFT of the Brazil strain of *T. cruzi*. Data are shown as mean number of BFT per ml of blood. (RT = room temperature; DI = depleted infected mice; NI = normal IgG-treated infected mice).

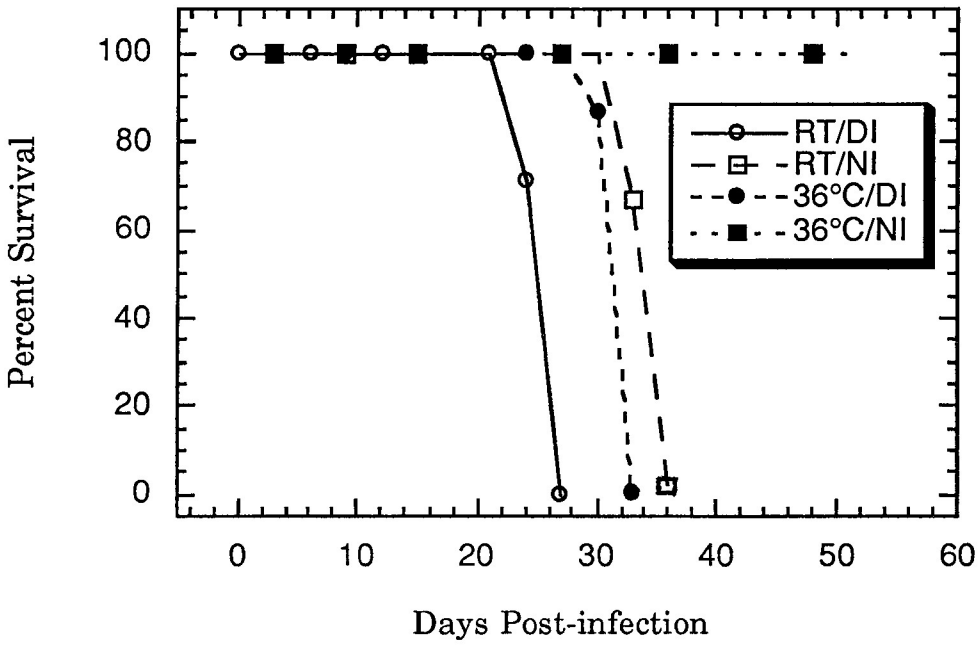

Figure 5. Effect of CD8+ T cell depletion on mortality. Effect of anti-CD8 and normal rat IgG treatment on percent survival of C3H mice maintained at either room temperature or 36°C. Two groups of five mice were injected with 200 μg anti-CD8 monoclonal antibody, and two groups of mice were injected with 200 μg normal rat IgG for three consecutive days. On day five, C3H mice were infected with 10^3 BFT of *T. cruzi.* Mortality was monitored during the course of infection. (RT = room temperature; DI = depleted infected mice; NI = normal IgG-treated infected mice).

DISCUSSION

The protective immune response to *T. cruzi* involves many aspects of immunity. Antibody dependent killing of extracellular parasites (Kretti and Brener, 1976; 1982), activated macrophage killing of intracellular parasites, and helper function of CD4+ T cells (Rottenberg et al., 1988; Araujo, 1989; Rottenberg et al., 1990) have been thought to be the primary mechanisms. The regulatory functions of IFN-γ (Reed et al., 1987; Reed, 1988) IL-2, IL-4 and IL-10 cytokines (Choromanski and Kuhn, 1985; Wirth et al., 1985; 1989; Silva et al., 1992) have been regarded as the major protective mechanisms. However, there has been increasing evidence that CD8+ T cells play a significant role in immunity to *T. cruzi* infection. The first evidence was provided by Tarleton that *in vivo* depletion of CD8+ T cells with anti-CD8 monoclonal antibody resulted in increased susceptibility to *T. cruzi* infection in both resistant and susceptible mouse strains (Tarleton, 1990). In addition, ß2-microglobulin deficient mice experienced high parasitemia levels and early death when infected with *T. cruzi* (Tarleton, 1992). The antigen-specific cytotoxic activity of CD8+ T cells directed against *T. cruzi* infected target cells was confirmed by Nickell (1993). Splenic lymphocytes from *T. cruzi* infected mice were shown to have high levels of cytotoxicity when stimulated *in vitro* with irradiated *T.*

cruzi-infected macrophages.

The results of the present study confirm these earlier studies demonstrating a requirement for CD8+ T cells in protective immunity against *T. cruzi* in mice held at room temperature. In addition, CD8+ T cells were also found to be necessary for the enhanced survival of *T. cruzi*-infected C3H mice maintained at elevated temperature. Anti-CD8 MAb treated infected mice showed higher parasitemia levels and earlier death than normal IgG-treated infected mice held at either temperature.

Elevated environmental temperature can enhance immunity to parasitic infection through effects on both the host and the parasite. Several investigators have reported that *T. cruzi*-infected mice survive an otherwise lethal infection when maintained at elevated environmental temperature (Amrein, 1967; Anderson and Kuhn, 1989; Marinkelle and Rodriguez, 1968; Otieno, 1972; 1973, Trejos et al., 1965). The effect of elevated environmental temperature on the immune response to *T. cruzi* in infected mice is reflected not only by decreased parasitemia levels and increased longevity but also by enhanced antibody responses (Marinkelle and Rodriguez, 1968; Smith et al., 1978; Saririan and Nickerson, 1982; Jampel et al., 1983; Anderson and Kuhn, 1989; Dimock et al., 1991). It has been reported that effects of elevated environmental temperature on parasites may include the production of heat shock proteins (HSPs) (Engman et al., 1990). The HSPs can serve as targets of the immune response; also, since there is a high degree of sequence homology between host HSPs and parasite homologies, parasite HSPs may induce autoimmune phenomena.

HSP 70 of *T. cruzi* was reported to be a major target of antibodies in infected persons (Newport et al., 1988; Mattei et al., 1989).

The cellular immune response during *T. cruzi* infection in mice held at elevated environmental temperature has not been well studied. The results reported in this thesis provide the first evidence for a protective role of CD8+ T cells in the immune response to *T. cruzi* in mice held at elevated environmental temperature. The specific mechanism by which CD8+ T cells control parasitemia and mortality are not known. Protection is most likely due to the cytotoxic function of CD8+ T cells (Tarleton, 1990; 1992; Nickell, 1993), but could possibly be due to enhanced production of IFN-γ (Reed et al., 1987; Reed, 1988; Tarleton, 1990) or IL-2 (Choromanski and Kuhn, 1985; Wirth et al., 1985). As expected, normal rat IgG-treated infected mice held at elevated environmental temperature survived infection with *T. cruzi* and experienced very low parasitemia levels. However, anti-CD8 treated infected mice maintained at 36°C were unable to survive infection with *T. cruzi.* Anti-CD8 treated infected C3H mice held at 36°C did experience a delay in the appearance of parasites in the blood as compared to anti-CD8 treated mice maintained at room temperature. In addition, parasitemia levels were slightly lower and mice survived for a longer period of time when depleted of CD8+ T cells and maintained at elevated temperature. Anti-CD8 treated infected mice held at 36°C died approximately 1 week later than those held at room temperature. These studies suggest that there may be a slight direct effect of elevated

temperature on the parasites. Similarly, Dimock and coworkers (1991) reported that when mice held at 36°C were treated with the immunosuppressive agent cyclophosphamide, peak mean parasitemia levels were 8.6-fold greater than those of non-cyclophosphamide-treated mice held at 36°C. In addition, the cyclophosphamide-treated mice all died by day 31 of infection. However, cyclophosphamide-treated mice held at room temperature developed slightly higher parasitemia levels and died by day 27 of infection. It has also been reported that cyclophosphamide-treatment of *Trypanosoma brucei*-infected mice held at 35°C abrogated the beneficial effects of elevated environmental temperature (Otieno, 1973). In addition, protection observed in *T. cruzi*-infected mice held at 36°C could be conferred on infected mice held at room temperature by passive transfer of hyperimmune sera (Dimock et al., 1991). These results suggest that although there may be some direct effect of elevated temperature on the parasite, the primary beneficial effect of elevated environmental temperature on *T. cruzi* infection in mice is the enhancement of parasite-specific immunity.

Based upon the results presented in this study, it is evident that CD8+ T cells are required in the protective immune response to *T. cruzi* in infected mice held at elevated environmental temperature. In the absence of a cytotoxic T cell response, mice are unable to survive infection with the parasite.

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